

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1. (Original): A method for determining whether an activity of an enzyme selected from the group consisting of a sphingosine kinase and a phosphatase involved in the sphingolipid pathway is present in a sample or not, or determining the extent of said activity comprising the steps of either

- a. contacting living cells comprised in an appropriate culture medium with a labeled sphingosine for a predetermined period of time so that an enzymatic product can be formed,
 - b. separating the enzymatic product formed in step a., and
 - c. determining the amount of enzymatic product formed or
- for determining whether an activity of a sphingosine kinase is present in a sample or not, or determining the extent of said activity comprising the steps of
- A. contacting a labeled unphosphorylated sphingosine with
 - a sample which sample optionally comprises a sphingosine kinase and
 - a phosphate source,for a predetermined period of time so that an enzymatic product can be formed,
 - B. adding to the mixture of step A. an aqueous buffer solution and organic solvent which is able to form two phases in combination with water,
 - C. separating the phases obtained in step B,
 - D. determining the amount of enzymatic product in the aqueous phase obtained in step C..

Claim 2. (Original): A method for identifying an agent that modulates the activity of a sphingosine kinase comprising the steps of

- a. contacting a labeled unphosphorylated sphingosine with
 - a phosphate source, and
 - a sphingosine kinasefor a predetermined period of time so that an enzymatic product can be formed,
- a1. in the absence of a candidate compound, and
- a2. in the presence of a candidate compound,
- b. adding to the mixture of step a1 and of step a2 an aqueous buffer solution and organic solvent which is able to form two phases in combination with water,
- c. separating the unreacted labeled sphingosine from the enzymatic product formed in steps a1. and a2., e.g. according to claim 1, steps b. and c.,

- d. detecting the amount of enzymatic product obtained in step a1. and in step a2 and determining whether there is a difference in the amount of enzymatic products formed in step a1. and step a2.,
- e. choosing an agent that modulates the activity of a sphingosine kinase as determined in step d.

Claim 3. (Original): A method for identifying an agent that modulates the activity of a phosphatase involved in the sphingolipid pathway comprising the steps of

- A. contacting a labeled phosphorylated sphingosine with living cells comprised in an appropriate medium for a predetermined period of time so that an enzymatic product can be formed,
- A1. in the absence of a candidate compound, and
- A2. in the presence of a candidate compound,
- B. separating the unreacted labeled phosphorylated sphingosine from the enzymatic product formed in steps A1. and A2.,
- C. detecting the amount of enzymatic product obtained in step A1. and in step A2 and determining whether there is a difference in the amount of enzymatic products formed in step A1. and step A2.,
- D. choosing an agent that modulates the activity of a phosphatase involved in the sphingolipid pathway as determined in step C.

Claim 4. (Original): A method for determining whether in a sample sphingosine kinase-1-activity or sphingosine kinase-2-activity or both or no sphingosine kinase activity is present comprising the steps of

- α. contacting
 - α1. a labeled unphosphorylated sphingosine with a sample which sample optionally comprises sphingosine kinase-1-activity, or sphingosine kinase-2-activity, or both, or no sphingosine kinase activity, with a phosphate source,
 - α2. a labeled unphosphorylated sphingosine with a sample comprising a defined amount of sphingosine kinase-1-activity with a phosphate source,
 - α3. a labeled unphosphorylated sphingosine with a sample comprising a defined amount of sphingosine kinase-2-activity with a phosphate source for a predetermined period of time so that an enzymatic product can be formed,
- β. separating the unreacted compound of a labeled sphingosine from the enzymatic product formed in steps α1., α2. and α3., e.g. according to method steps b. and c. as defined in claim 1, and
- γ. determining and comparing the phosphate conversion rate in steps α1., α2. and α3.

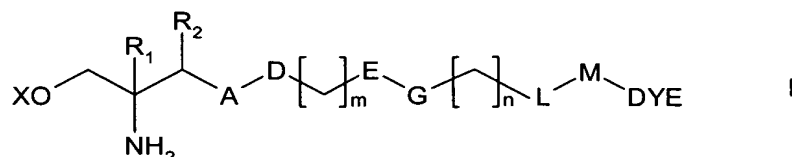
Claim 5. (Original): A method for differentiating whether a test compound is capable to mediate the activity of a sphingosine kinase-1 and/or a sphingosine kinase-2 comprising the steps

- i. contacting an unphosphorylated compound of formula I with a phosphate source and with
 - i1. a sphingosine kinase-1,
 - i2. a sphingosine kinase-2,
 - in the absence of a test compound, and
 - in the presence of a test compound
 for a predetermined period of time so that an enzymatic product can be formed,
- ii. separating the unreacted unphosphorylated compound of formula I from the enzymatic product formed in steps i1. and i2., e.g. according to method steps b. and c. as defined in claim 1, and
- iii. determining and comparing the phosphate conversion rate in steps i1. and i2..

Claim 6. (Original): A kit for kit for determining the activity of an enzyme selected from the group consisting of a sphingosine kinase and a phosphatase involved in the sphingolipid pathway comprising as a main component a labeled sphingosine and instructions for using said kit.

Claim 7. (Original): A kit of claim 6 for use in the identification of an agent that mediates the activity of an enzyme selected from the group consisting of a sphingosine kinase and a phosphatase involved in the sphingolipid pathway.

Claim 8. (Currently amended): ~~The use, the method of any one of claims 1 to 5, or a kit of an one of claims 6 or 7~~ wherein the labeled sphingosine is a compound of formula



wherein

R₁ is H or (C₁₋₄)alkyl,

R₂ is H, OH or oxo,

X is H or (HO)₂PO,

A-D, E-G and L-M independently of each other is a group

CH₂-CH₂, CH=CH, C≡C, CH₂-phenyl, phenyl-CH₂, CH₂-CH₂-phenyl,

CH₂-NH, CH₂-N((C₁₋₄)alkyl), NH-CH₂, N((C₁₋₄)alkyl)-CH₂, O-CH₂, CH₂-O, phenyl-O, O-phenyl,

CH₂-phenyl-O, O-CO, CO-O, CO-NH, NH-CO, CO-N((C₁₋₄)alkyl), N(C₁₋₄)alkyl-CO, NH-SO₂,

SO₂-NH, N((C₁₋₄)alkyl)-SO₂,

or one group out of A-D, E-G and L-M is absent

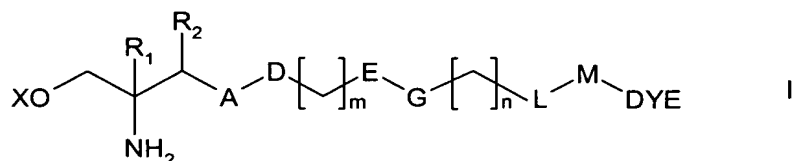
m is a number selected from 0 to 12,

n is a number selected from 0 to 12,

and m plus n is a number selected from 0 to 14,

the group DYE is a group selectively detectable in a compound of formula I by physical means, with the proviso that at least one of E-G and L-M is selected from the group consisting of CH₂-NH, CH₂-N((C₁₋₄)alkyl), CH₂-O, phenyl-O, O-CO, CO-O, CO-NH, NH-CO, CO-N((C₁₋₄)alkyl), N(C₁₋₄)alkyl-CO, NH-SO₂, N((C₁₋₄)alkyl)-SO₂.

Claim 9. (Original): A compound of formula



wherein

R₁ is H or (C₁₋₄)alkyl,

R₂ is H, OH or oxo, e.g. H or OH,

X is H or (HO)₂PO,

A-D, E-G and L-M independently of each other is a group

CH₂-CH₂, CH=CH, C≡C, CH₂-phenyl, phenyl-CH₂, CH₂-CH₂-phenyl,

CH₂-NH, CH₂-N((C₁₋₄)alkyl), NH-CH₂, N((C₁₋₄)alkyl)-CH₂, O-CH₂, CH₂-O,

phenyl-O, O-phenyl, CH₂-phenyl-O, O-CO, CO-O, CO-NH, NH-CO, CO-N((C₁₋₄)alkyl),

N(C₁₋₄)alkyl-CO, NH-SO₂, SO₂-NH, N((C₁₋₄)alkyl)-SO₂,

or one group out of A-D, E-G and L-M is absent,

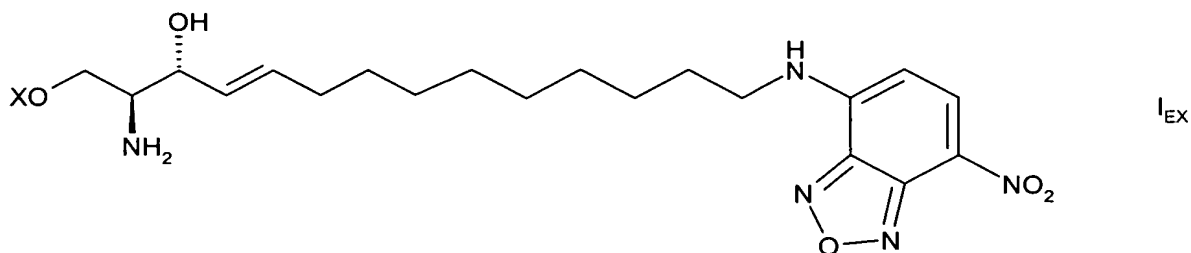
m is a number selected from 0 to 12,

n is a number selected from 0 to 12,

m plus n is a number selected from 0 to 14, and

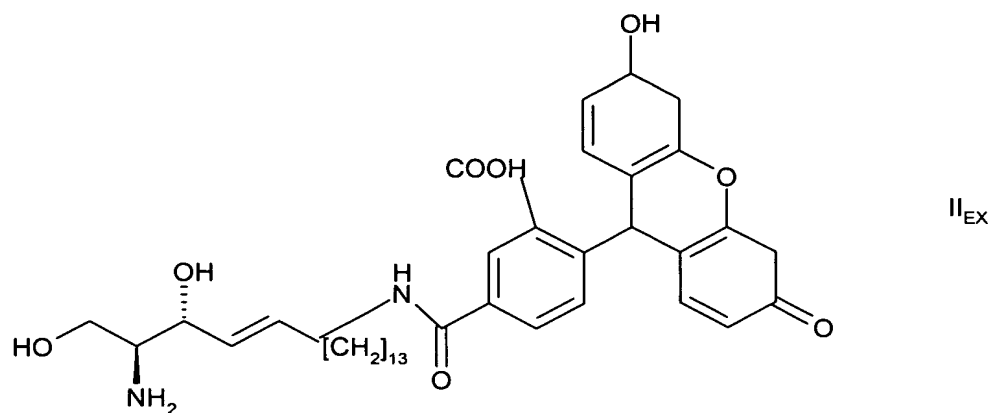
the group DYE is a group selectively detectable in a compound of formula I by physical means, with the proviso that

- at least one of E-G and L-M is selected from the group consisting of CH₂-NH, CH₂-N((C₁₋₄)alkyl), CH₂-O, phenyl-O, O-CO, CO-O, CO-NH, NH-CO, CO-N((C₁₋₄)alkyl), N(C₁₋₄)alkyl-CO, NH-SO₂, N((C₁₋₄)alkyl)-SO₂,
- a compound of formula



wherein X is as defined above, and

- a compound of formula



are excluded.

Claim 10. (Original): The use of a fluorescent labeled sphingosine of formula I as defined in claim 9 in a high-throughput assay, e.g. for the identification of an agent that modulates the activity of an enzyme selected from the group consisting of a sphingosine kinase and a phosphatase involved in the sphingolipid pathway.

Claim 11. (Currently amended): An agent which is capable to mediate an enzyme selected from the group consisting of a sphingosine kinase and a phosphatase involved in the sphingolipid pathway, which agent is identified by a method of ~~any one of claims 2 or 3.~~